CUTANEOUS AND PULMONARY GAS EXCHANGE IN THE SPOTTED SALAMANDER, AMBYSTOMA MACULATUM [1]

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The first quantitative study of pulmonary and cutaneous respiration in amphibians was conducted on *Rana esculenta* and *R. fusca* by Krogh (1904). He inserted a cannula, connected to an air pump, into the trachea and analyzed separately the air forced through the lungs by the pump and the air surrounding the frog. Krogh found that carbon dioxide was released chiefly through the skin, while oxygen was taken up predominantly by the lungs. He also found that oxygen uptake through the skin remained relatively constant throughout the year, while oxygen uptake by the lungs was greatest during the spring and dropped below cutaneous uptake during the fall and winter. His curves for release of carbon dioxide through the skin and lungs followed the pulmonary oxygen uptake curve throughout the year. Dolk and Postma (1927), using similar techniques with *Rana temporaria,* substantiated Krogh's results.

Lapicque and Petetin (1910) demonstrated that cutaneous respiration in the lungless salamander, *Euproctus montanus,* may be more important than lung and/or buccopharyngeal respiration. They found that *E. montanus* dies quickly when submerged in Vaseline with its head free, but can live without buccopharyngeal respiration. However, their study did not solve the problem of the relative importance of cutaneous and buccopharyngeal or pulmonary respiration in salamanders.

Since the relative roles of pulmonary and cutaneous respiration in salamanders had not been studied quantitatively, we undertook the present study to determine the role of each in the respiration of *Ambystoma maculatum.*

Methods and Materials

The animals used in this study were collected in late March, 1962, in the vicinity of Kingston, R. I. Groups of animals were acclimated in constant temperature chambers in total darkness. The minimum standards used for acclimation were as follows:

5° C. - - - one week at 15° C.; two weeks at 10° C.; 10 days at 5° C.
10° C. - - - one week at 15° C.; two weeks at 10° C.
15° C. - - - one week at 15° C.
25° C. - - - one week at 15° C.; one week at 25° C.
30° C. - - - one week at 15° C.; one week at 25° C.; 5 days at 30° C.

A mask of 0.5-inch Tygon flexible plastic tubing was sutured to the head of the animal at least 24 hours prior to use. The mask was constructed in a manner that

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would not interfere with normal buccal movements (Fig. 1). Pulmonary and cutaneous respiration were determined in a closed system respirometer.

The respirometer consisted of four chambers of equal volume constructed of 0.25-inch acrylic plastic (Fig. 2). The two rear chambers served as thermobarometers, while pulmonary and cutaneous respiration were measured separately and simultaneously in the two front chambers, which were connected by a 0.5-inch hole. A cover of 0.25-inch plastic was screwed down over the four chambers and sealed with petroleum jelly. A series of plastic connectors with stopcocks communicated through the cover into the respiration chambers. Manometers with colored kerosene indicators were fitted into the stopcock connectors. Syringes filled with 100% oxygen were fitted to the stopcock connectors of the pulmonary

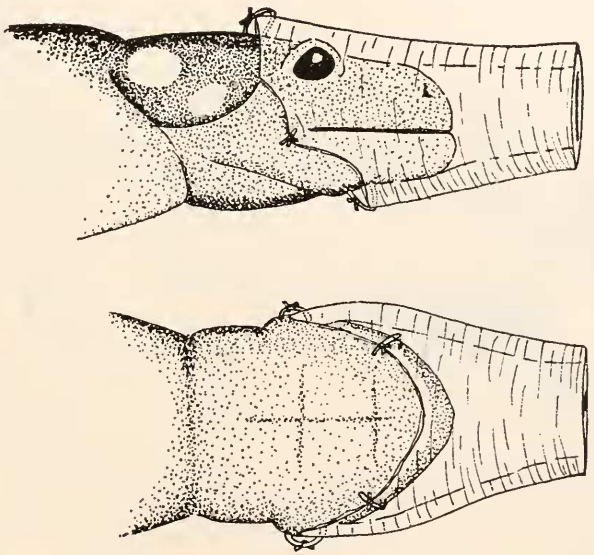

FIGURE 1. Method of attaching plastic mask to salamanders.

and cutaneous chambers. All connections were checked for leaks prior to each set of experiments.

The respirometer was constructed within a larger plastic chamber which served as a water jacket. A cooling coil, heating coil, temperature regulator, and stirrer were placed in the water jacket for temperature control. The temperature-regulating system kept the water temperature constant within $\pm 0.1^\circ$ C.

The masked animal was tied firmly to a piece of hardware cloth and the mask fitted through the hole between the chambers. The end of the mask was sealed into the hole in the chamber wall by the application of petroleum jelly. Beakers containing 10 ml. of barium hydroxide were placed in each chamber to absorb carbon dioxide. The beakers of barium hydroxide contained plastic-coated magnetic bars which were moved at regular intervals by a magnet outside the respirometer chambers. This stirred the barium hydroxide solution and insured an effective absorption of the carbon dioxide by breaking the barium carbonate

film which formed on the surface and which would have resulted in reduced absorption of carbon dioxide. Oxygen injected into the chambers by the syringes compensated for oxygen consumed by the animal. Oxygen consumption was read directly from the calibrated syringes.

At the end of a set of experiments, the beakers of barium hydroxide were removed from the chambers and titrated with standardized 1 N sulfuric acid to determine the quantity of carbon dioxide produced. The beakers of barium hydroxide in the thermobarometers served as controls, since each beaker of barium hydroxide absorbed carbon dioxide at the same rate both prior to the experiment and during the time required for titration. To determine the actual amounts of carbon dioxide released by the animal, the amount of carbon dioxide absorbed in the

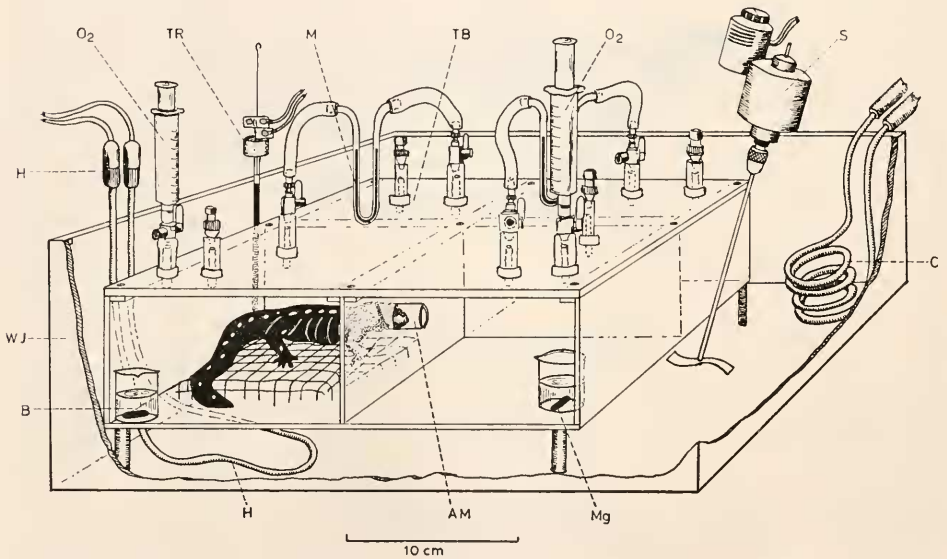

FIGURE 2. Apparatus used to measure simultaneously pulmonary and cutaneous respiration in amphibians. AM, animal mask; B, barium hydroxide solution in beaker; C, cooling coil; H, heating coil; M, manometer; Mg, magnetic stirring bar; O_2, oxygen syringe; TB, thermobarometer chamber; TR, temperature regulator; S, stirrer; WJ, water jacket.

thermobarometers was subtracted from the amounts of carbon dioxide absorbed in the pulmonary and cutaneous chambers.

The few animals that struggled against their bonds during the first hour of the experiment produced high oxygen consumption values. The values obtained for these hours were not included in the calculations of mean oxygen consumption, but the carbon dioxide produced had to be included in the determination of respiratory quotients, since the barium hydroxide could not be removed for titration after each hour.

Measurements of total oxygen consumption were made as controls. Differences in oxygen consumption between masked and unmasked animals were not statistically significant. Oxygen consumption measurements for masked and unmasked animals tied to hardware cloth indicated that restraint of the animals resulted in a

slight increase in oxygen consumption. Total oxygen consumption for experimental animals was about 5% higher than unrestrained controls at all temperatures except 5° C., where movement was negligible. Respiratory quotient values for control animals were not significantly different from those of experimentals.

Tidal volumes were measured by connecting the animal's mask to a graduated manometer. The volume of air required to move the manometer column a distance equal to that moved by the breathing of the animal was taken as the tidal volume.

Recordings of breathing movements were obtained for masked and unmasked animals acclimated to 10° C., 15° C., and 25° C. by passing a loop of thread under

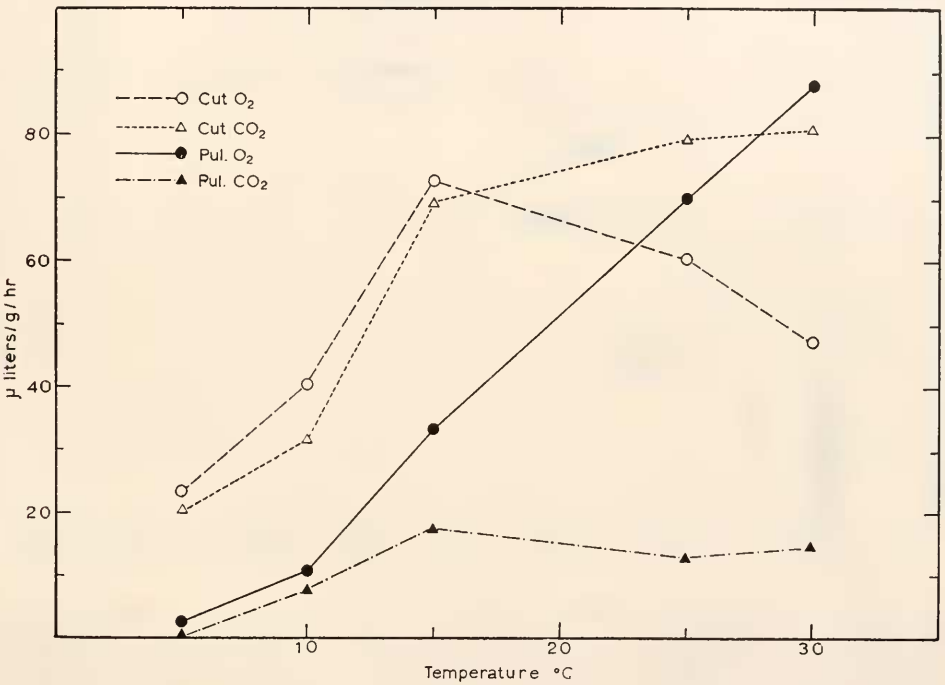

FIGURE 3. Mean cutaneous and pulmonary gas exchange at different temperatures.

the buccal floor of the animal and connecting it to a pressure transducer. Movement of the buccal floor caused movement of the transducer wire, which was connected to a Physiograph.

RESULTS

Pulmonary and cutaneous gas exchange

Pulmonary oxygen consumption increased almost linearly from 1.36 μl./gm./hr. at 5° C. to 86.59 μl./gm./hr. at 30° C. Cutaneous oxygen increased from a mean of 22.74 μl./gm./hr. at 5° C. to 73.00 μl./gm./hr. at 15° C., then dropped to 46.62 μl./gm./hr. at 30° C. (Figs. 3 and 4). The ratio of pulmonary to cutaneous

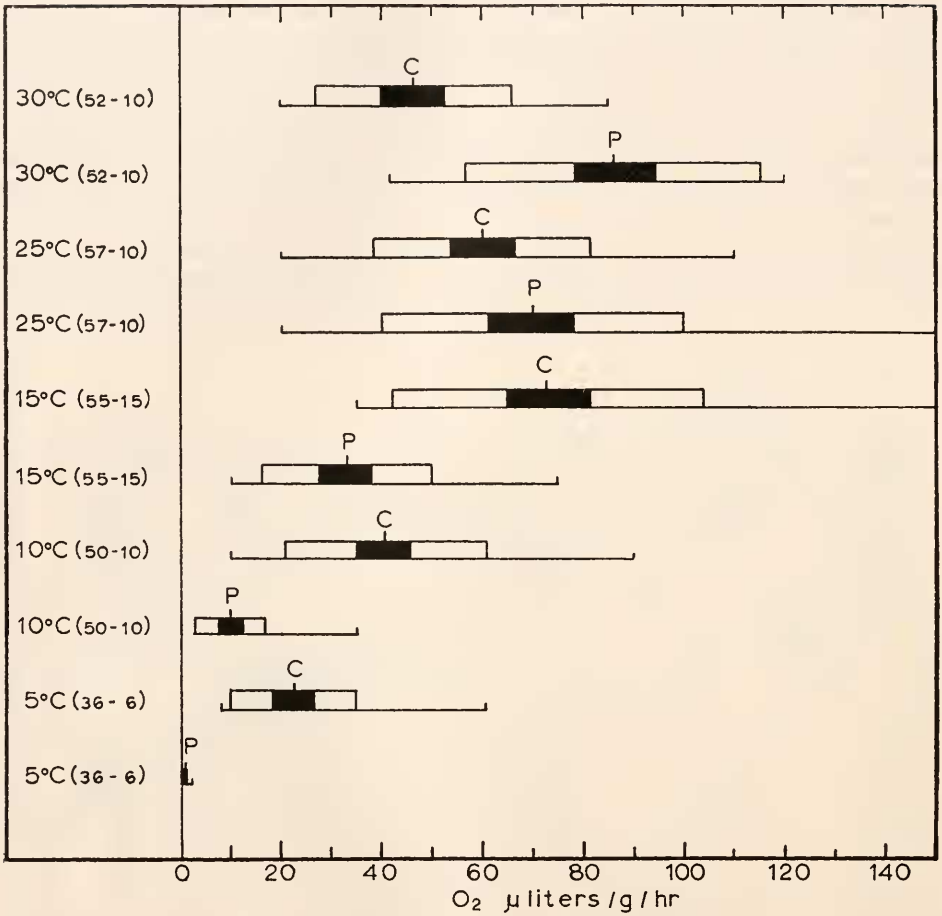

FIGURE 4. Cutaneous and pulmonary oxygen consumption at different temperatures. The first figure in parentheses indicates total number of hours of measurement; the second figure denotes number of individuals in sample. The horizontal line indicates the range, the thin vertical line, the mean; one black and one white rectangle combined on each side of the mean, one standard deviation; one black rectangle on each side of the mean, two standard errors. If the standard errors of two sets of data do not overlap, the difference between the means may be considered statistically significant (Hubbs and Hubbs, 1953).

oxygen consumption increased with temperature (Fig. 5). No measureable amount of carbon dioxide was released through the lungs and buccopharyngeal mucosa at 5° C. A sharp increase in both pulmonary and cutaneous carbon dioxide occurred between 10° and 15° C. At temperatures above 15° C. pulmonary carbon dioxide remained almost constant (Figs. 3 and 6). Cutaneous carbon dioxide increased gradually with increasing temperature. The ratio of pulmonary to cutaneous carbon dioxide release was approximately 0.2 at all temperatures except at 5° C., where there was no measureable release of carbon dioxide from the lungs and buccopharyngeal surfaces.

Respiratory quotients

The range and mean of respiratory quotients, (RQ), were: 5° C. 0.73–0.81, $\bar{x} = 0.76$; 10° C., 0.71–0.85, $\bar{x} = 0.77$; 15° C., 0.72–0.89, $\bar{x} = 0.78$; 25° C., 0.70–0.87, $\bar{x} = 0.76$; 30° C., 0.71–0.88, $\bar{x} = 0.77$. Animals in acclimation were fed a diet of mealworms at regular intervals; those that refused to eat had RQ values between 0.70 and 0.72. In animals kept at 30° C., the mean RQ changed from 0.82 one to three days after feeding, to 0.72 five days after feeding. For animals acclimated to 10°, 15°, and 25° C., RQ's between 0.84 and 0.80, observed

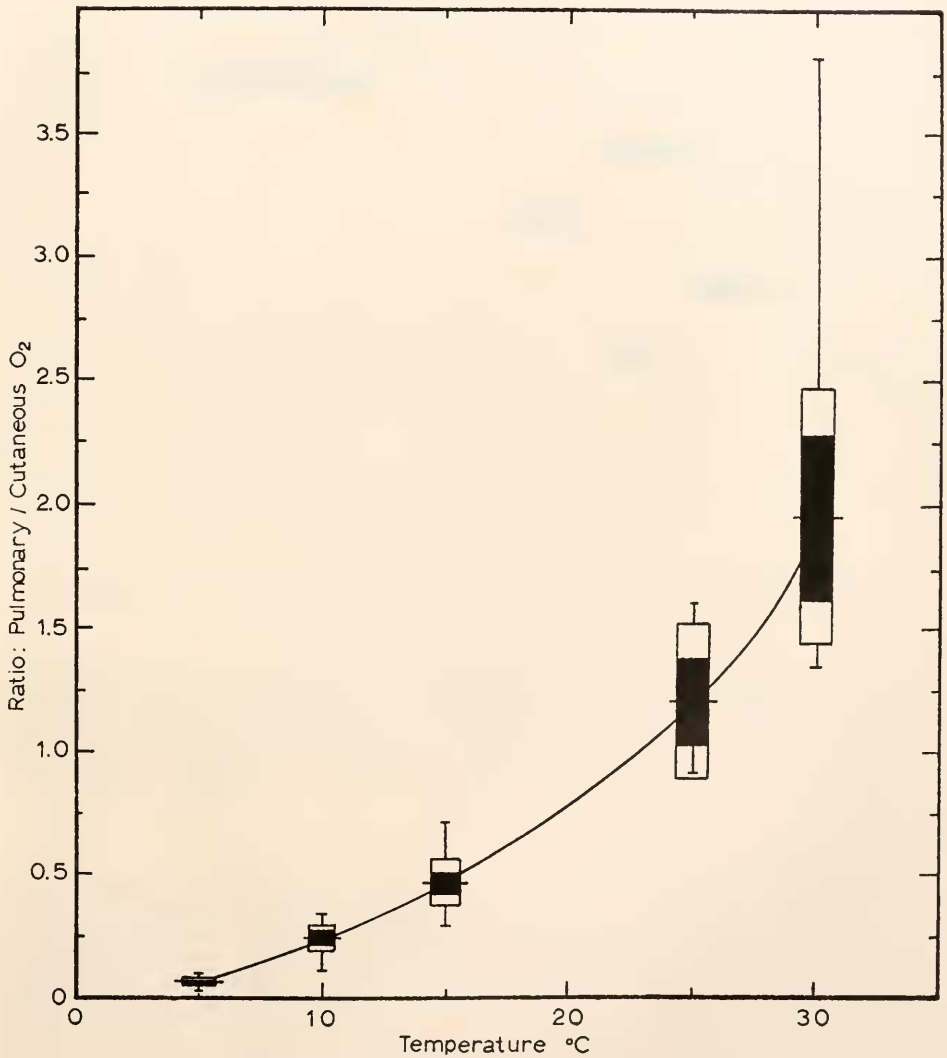

FIGURE 5. The ratio of pulmonary to cutaneous oxygen consumption at different temperatures. Method of presentation is the same as in Figure 4.

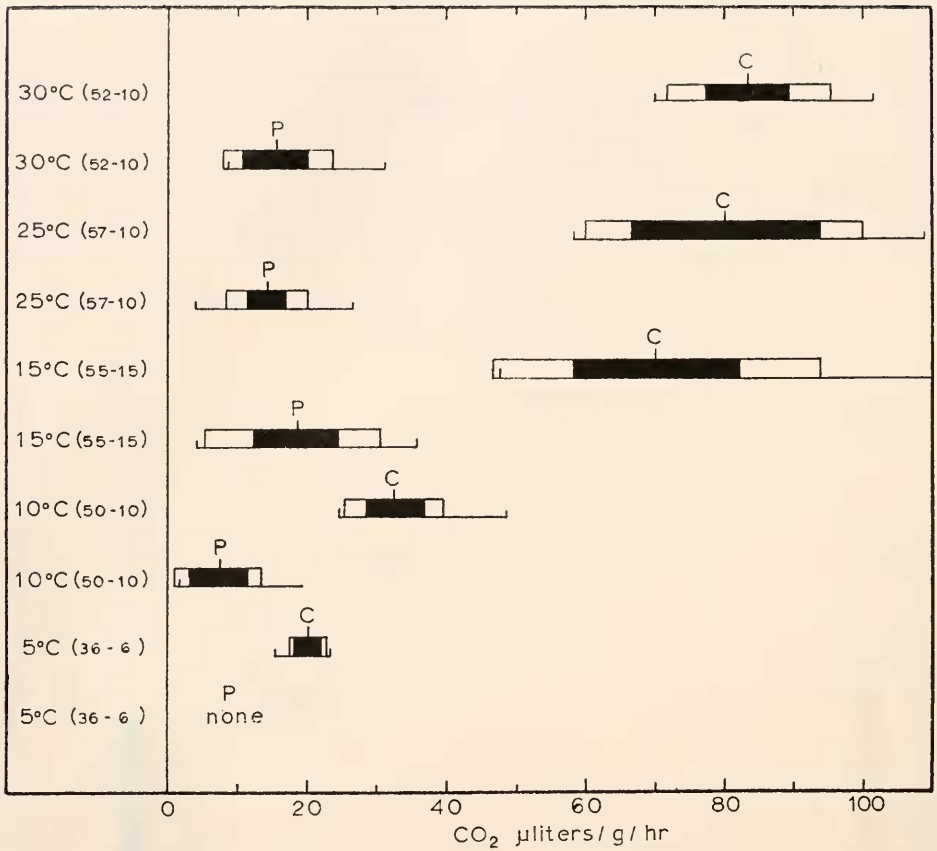

FIGURE 6. Cutaneous and pulmonary carbon dioxide release at different temperatures. Method of presentation is the same as in Figure 4.

from one to four days after feeding, decreased to 0.72 eight days after feeding. At 5° C., mean RQ's increased from 0.74 three days after feeding, to 0.78 five days after feeding. Meal worms could be detected in the stomachs of animals acclimated to 5° C., up to five days after feeding, indicating incomplete digestion and assimilation.

Tidal volumes and breathing rates

Two distinct breathing movements, including two separate tidal volumes, occur in salamanders with lungs. The buccopharyngeal movement consists of an enlargement of the buccopharyngeal cavity by a lowering of the hyobranchial apparatus, resulting in the inspiration of air through the nares. Exhalation occurs when the buccal floor rises again. A pronounced depression of the buccal floor occurs at intervals. During the latter part of this depression, the nares completely close, the buccal floor raises and forces the air into the lungs (Whipple, 1906).

Temperature had a direct effect on tidal volumes (Fig. 7). The mean buc-

copharyngeal tidal volume increased from 0.008 cc. at 5° C. to 0.065 cc. at 25° C. and 30° C. The mean lung tidal volume increased from 0.09 cc. at 5° C. to 0.42 cc. at 30° C.

The number of deep inspirations, *i.e.*, those movements in which air was forced into the lungs, remained relatively constant (between six and nine per minute at

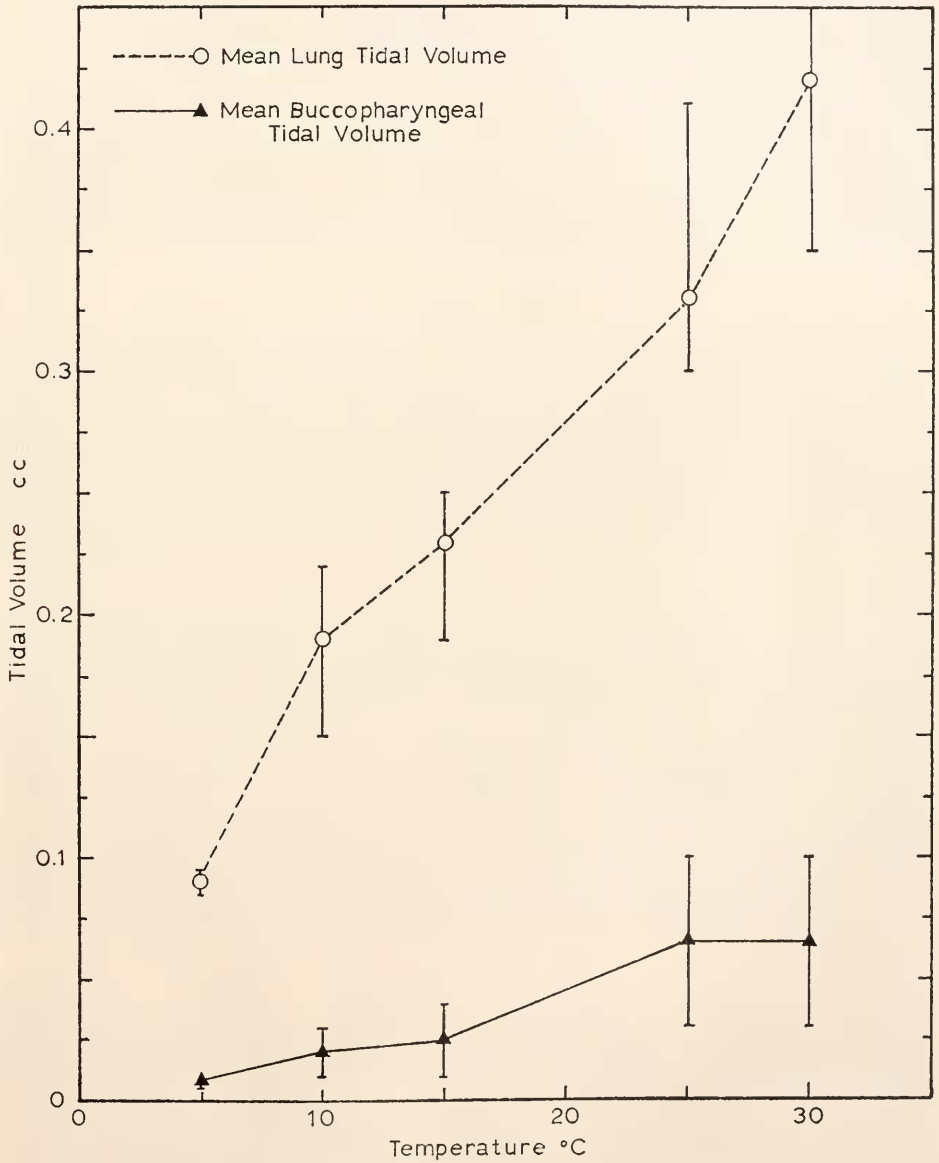

FIGURE 7. Mean lung and buccopharyngeal tidal volumes at different temperatures. Vertical lines denote the range of observed variation.

all temperatures). However, the rate of buccopharyngeal oscillations was dependent upon temperature. The average rate of buccopharyngeal movements was 13 at 10° C., 80 at 15° C., and 91 at 25° C. Masking had no visible effect on breathing rates.

The mean volume of air inspired by buccopharyngeal movements at 10° C., was 15.6 cc. per minute; by lung inspirations, 68.4 cc. per minute; but at 25° C. the mean volume of air inspired by buccopharyngeal movements was 382.2 cc. per minute and by lung inspirations, 194.4 cc. per minute. Thus, the volume of air moved by buccopharyngeal oscillations at 25° C. is approximately 25 times that moved at 10° C., while that moved by the lungs is only about three times as great.

The per cent efficiency of the lung-buccopharyngeal mucosa combination was calculated by dividing the oxygen consumption per hour by the total amount of oxygen inspired, the total volume of inspired air being calculated from breathing rates and tidal volumes. The following are the calculated efficiencies: 10° C., 1.43%; 15° C., 0.93%; 25° C., 0.86%.

Discussion

Krogh (1904) and Dolk and Postma (1927) probably introduced experimental error into their measurements of lung respiration by failure to acclimate their experimental animals adequately to constant temperature. In addition, they cannulated the trachea and forced air in and out of the lungs with a mechanical pump, a procedure that probably does not duplicate the two distinct breathing movements in frogs (Cole and Allison, 1929; Scholten, 1942). The mask used in our experiments did not affect either the breathing movements or the breathing rates of the experimental animals.

With increasing environmental temperatures, the lungs and buccopharyngeal mucosa play an increasing role in oxygen uptake in *A. maculatum*. Since oxygen uptake through the skin is passive, it is dependent upon the proximity of the capillaries to the surface of the skin, the rate of blood flow through the capillaries, and the affinity of the hemoglobin for oxygen. Uptake of oxygen through the lungs and buccopharyngeal mucosa is not only dependent on these same factors but also on the depth and rate of breathing movements. Therefore, the increase in oxygen uptake through the lungs and buccopharyngeal mucosa that occurs with rising temperature can be directly correlated with increases in tidal volumes and in breathing rates. At the same time, the rate of lung inspirations remains relatively constant, changing from six at 10° C. to nine at 25° C., while the rate of buccopharyngeal oscillations increases greatly, from 13 per minute at 10° C. to 91 per minute at 25° C.

Matthes (1927), Vos (1936) and Elkan (1955) concluded that the buccopharyngeal oscillations of amphibians were olfactory in function, while Noble (1925) had assumed that these movements were primarily for respiration. Czopek (1962) found that the capillaries of the mouth cavity in *A. opacum* accounted for only 6.4% of the respiratory capillaries and concluded that (p. 586), "the pulsation of the buccal floor . . . is probably connected with olfactory functions rather than respiration." He pointed out, however, that (p. 586) "conclusions derived exclusively from morphological findings must be accepted with prudence

unless they are supported by physiological investigations." Our data indicate that buccopharyngeal movements are of appreciable value in respiration, especially at higher temperatures. Between 10° C. and 25° C., the volume of air moved through the lungs increases about three-fold; the volume of air moved through the buccopharyngeal cavity increases 25-fold. If the air moved by the buccopharyngeal oscillations were excluded from respiration, the efficiency of the lungs would have to double to account for the increased oxygen consumption at 25° C.

In nature during the warmer months of the year, *A. maculatum* would probably have food in its stomach three to five days after feeding. At normal environmental temperatures (10° C. to 25° C.), five to six days are necessary for complete digestion and assimilation of food because during this period RQ values remain above fasting levels.

Cutaneous oxygen uptake increased linearly between 5° C. and 15° C., but dropped to lower values at 25° C. and 30° C. This decrease in oxygen uptake through the skin at temperatures above 15° C. may be due to several factors. *A. maculatum,* in its natural environment, remains burrowed in moist leaf litter or rotten logs, coming to the surface to feed at night, and is most active on rainy nights. In this micro-environment, this species rarely encounters temperatures exceeding 20° C. It is possible, therefore, that during its evolutionary history, certain enzymes or other physiological systems became adapted to function optimally at temperatures approximating 15° C. to the point where they are not sufficiently labile to be altered significantly by changes in acclimation temperatures. If enzyme systems of *A. maculatum* are adjusted to function optimally at temperatures approximating 15° C., higher temperatures could result in decreased oxygen uptake through the skin.

Summary

1. In *Ambystoma maculatum,* the lungs and buccopharyngeal mucosa become increasingly important in respiration at higher temperature.

2. The skin accounts for more than 50% of the total oxygen uptake at 15° C. and below.

3. Approximately 80% of the carbon dioxide produced is released through the skin at all temperatures except 5° C., where no measureable amount of carbon dioxide is released through the lungs and buccopharyngeal mucosa.

4. Lung and buccopharyngeal tidal volumes increased directly with temperature; and the rate of buccopharyngeal oscillations increased greatly at higher temperatures, while the rate of lung inspirations remained relatively constant.

5. Buccopharyngeal oscillations are of appreciable importance in the respiration of *A. maculatum,* especially at higher temperatures.

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